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## **GB Toxicity in Mice Exposed for 20 to 720 Min.**

R.W. Bide and D.J. Risk  
Defence R&D Canada – Suffield

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DRDC Suffield TR 2002-031  
October 2002

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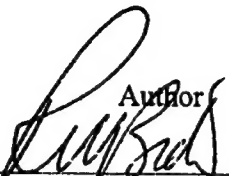
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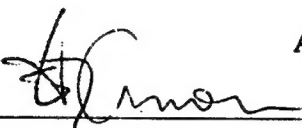
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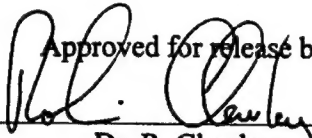
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**DRDC Suffield Animal Care Statement**

In conducting the research described in this report, the investigators adhered to the "Guide to the Care and Use of Experimental Animals, Vol. I, 2<sup>nd</sup> Ed." published by the Canadian Council on Animal Care.

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## Abstract

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Most of the historical data for the toxicity of GB was collected for exposure times <10 min in attempts to establish the utility of and defence against this agent in offensive front line use. However, information concerning the toxicity of GB (and other nerve agents) from longer exposures of one to twelve hours is critical for personnel functioning for long periods in collective protection, for flight crew waiting for action in areas of low level contamination, for aircrew transporting previously contaminated material in cargo bays, for hospital staff who must work with contaminated victims and finally for all personnel that have been attacked, are dressed in Individual Protective Equipment and need to know when, and if, it is safe to take off these cumbersome garments.

The data presented for the toxicity of GB to mice for exposures of 20 min to 12 hours are intended to form part of an international, collaborative, multi-species effort aimed at establishing toxicity estimates for humans for these longer exposure times.  $LC_{t50}$  values of 430, 538, 899, 1209 and 2214  $mg \cdot min/m^3$  were obtained for mice for 20, 60, 180, 360 and 720 min exposures to GB, respectively. The 20 and 60 min data fit well with historical data for 20 sec to 30 min exposures. The data for longer exposures do not follow either Haber's rule ( $LC_{t50} = CT$ ) or the "toxic load" expression involving  $C^nT$  previously established for 10 sec to 30 min exposures. The experimental data appear to indicate a threshold exposure concentration near 3  $mg/m^3$  for the  $LC_{50}$  for exposures up to 12 hr.

## Résumé

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La plupart des données historiques de la toxicité du GB ont été recueillies pour des durées d'exposition <10 min dans l'espoir d'établir leur utilité comme arme ainsi que les défenses contre cet agent lors des attaques en première ligne. L'information concernant la toxicité des GB (et autres agents neurotoxiques) est cependant critique pour le personnel fonctionnant pendant de longues périodes dans une situation collective; les équipages de conduite qui attendent leurs ordres dans des zones à niveau de contamination faible, le personnel navigant ayant transporté antérieurement des matériaux contaminés dans les soutes, le personnel des hôpitaux devant travailler avec des victimes contaminées et finalement tout le personnel victime d'une attaque; tous doivent porter un équipement personnel protecteur et ont besoin de savoir si et quand il est sécuritaire d'enlever ces vêtements encombrants.

Les données présentées au sujet de la toxicité du GB pour les souris lors d'expositions allant de 20 min à 12 heures sont destinées à faire partie d'un effort international de collaboration plurispécifique visant à établir des estimations de toxicité pour les humains durant ces durées d'exposition plus longues. Pour les souris, les valeurs  $CL_{t50}$  de 430, 538, 899, 1209 et de 2214  $mg \cdot min/m^3$  ont été obtenues pour des durées d'exposition au GB de 20, 60, 180, 360 et de 720 min, respectivement. Les données des 20 et des 60 min correspondent bien avec les données historiques pour des durées d'exposition de 20 sec à 30 min. Les données pour des durées plus longues d'exposition ne respectent ni la règle d'Haber ( $CL_{t50} = CT$ ) ni l'expression de la « charge toxique » comprenant notamment  $C^nT$  établies antérieurement pour des durées d'exposition allant de 10 sec à 30 min. Les données expérimentales semblent indiquer un seuil de concentration d'exposition proche de 3  $mg/m^3$  pour une  $CL_{50}$  de durées d'exposition allant jusqu'à 12 heures.

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## **Executive summary**

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### **Background**

When the nerve agents were studied after WWII, the experiments done to define toxicity were centred on exposures less than ten min and were designed to describe the offensive utility of and defence from these new agents in front line use. When the literature concerning nerve agents was re-examined in preparation for the 1991 Gulf War, one of the main data gaps identified was the absence of definitive information concerning the toxicity of the nerve agents at exposure times greater than 10 min. Information concerning the toxicity of CW agents at these longer exposure times is critical for personnel functioning in collective protection when contamination levels can be low and exposures of many hours are encountered, for flight crew waiting for action in areas with low level contamination, for aircrew when previously contaminated materiel is confined for long flights in cargo bays, for hospital staff who must work with contaminated victims (as demonstrated during a recent terrorist incident in Tokyo) and finally for all personnel that have been attacked, are dressed in Individual Protective Equipment and need to know when, and if, it is safe to take off these cumbersome garments. Further, the data impact upon the design of detection equipment, monitors, alarm systems and protective equipment of all kinds. This study is intended to form part of a TTCP program to obtain the missing toxicity data for exposures between 10 min and 12 hours.

### **Methods**

Mice (CD-1 strain; 25 - 30 g body weight) were exposed, in groups of 6, to GB gas at stated levels for one of 20, 60, 180, 360 or 720 min. The mortalities were counted and an  $LC_{50}$  was calculated for each exposure time. The surviving mice were monitored for 21-24 days and the clinical signs and body weights were recorded.

### **Results and Discussion**

The toxicities ( $LC_{50}$ ) of GB to mice exposed for 20, 60, 180, 360 and 720 min were estimated to be 430, 538, 899, 1209 and 720  $mg \cdot min/m^3$ , respectively. In terms of  $LC_{50}$ , the toxicity values would be 21.5, 9.0, 4.9, 3.4 and 3.1  $mg/m^3$ , respectively. The 20 and 60 min values were comparable to the values predicted by the toxic load model developed earlier for 10 sec to 30 min exposures. At longer exposure times the lethality values diverge from the toxic load model.

As the exposure time increased, the sequence of clinical signs - excess salivation (ptyalism), tremors, convulsions, flaccid paralysis and death, were the same for all exposure times. The onset and development of clinical signs were prolonged. No extended wasting was observed among the survivors, although there were significant decreases in body weight immediately after exposure. Recovery from the insult required 4 - 8 days and, in some animals, body weights were still reduced after 21 days albeit that the mice were growing and appeared normal.

The relationship between toxicity and longer exposure times is not a simple one. It is different from the empirical toxic load model demonstrated previously for exposures <30 min. The values obtained indicate a relative decrease in toxicity as the exposure time increases. The data would indicate a trend towards a single  $LC_{50}$  value near  $3 \text{ mg/m}^3$  as the exposure time was increased to 12 hr. This suggests that there may be a practical threshold for lethal effects at exposures up to 12 hr as the toxicity/exposure time/effect curve was approaching a constant value. However, as non-lethal effects were not studied, it is possible that other physiologic effects, like miosis, may follow a different pattern of responses as the exposure time increases.

## Conclusions

The inhalation toxicity (lethality) values for mice have been estimated for 20 to 720 min exposures to GB.

The apparent inhalation toxicity of GB for 60 to 720 min exposures does not follow either Haber's Rule<sup>1</sup> or the toxic load model which describes the effects between 0.17 and 30 min.

The lethal toxicity values of  $LC_{50}$  were changed only small amount as the exposure time extended from 360 to 720 min. If this effect proves similar in other species, it may indicate, that for many military purposes, there will be a threshold lethality value for GB.

Bide, R.W. and Risk, D.J. (2002). GB toxicity in mice exposed for 20 to 720 min. (DRDC Suffield TR 2002-031). Defence R&D Canada – Suffield.

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<sup>1</sup> Haber's Rule states that the  $LCt_{50} = CT$  where  $C$  is the concentration and  $T$  is the exposure time.

# Sommaire

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## Contexte

Quand les agents neurotoxiques ont été étudiés après la deuxième guerre mondiale, les expériences effectuées pour définir la toxicité étaient centrées sur des durées d'exposition inférieures à dix minutes. Cette recherche avait été conçue pour décrire leur utilité comme arme offensive et pour décrire aussi les moyens de défense contre ces nouveaux agents utilisés en première ligne. Quand la documentation concernant les agents neurotoxiques a été réexaminée, durant les préparatifs de la guerre du Golfe en 1991, une des lacunes les plus importantes des données a été identifiée comme étant l'absence d'information définitive au sujet de la toxicité des agents neurotoxiques pour des durées d'exposition supérieures à 10 minutes. L'information concernant la toxicité de ces agents chimiques de guerre durant ces expositions plus longues est critique pour le personnel fonctionnant en situation de protection collective; quand les niveaux de contamination restent faibles et que l'exposition dure plusieurs heures, les équipages de conduite attendent leurs ordres dans des zones ayant un niveau de contamination faible, le personnel navigant a antérieurement transporté des matériaux contaminés dans les soutes, pendant de longs vols, le personnel des hôpitaux doit travailler avec des victimes contaminées (comme il a été démontré récemment durant un incident terroriste à Tokyo) et finalement tout le personnel victime d'une attaque; tous doivent porter un équipement personnel protecteur et ont besoin de savoir quand et s'il est sécuritaire d'enlever ces vêtements encombrants. De plus, ces données ont un impact sur la conception de l'équipement de détection, des moniteurs, des systèmes d'alarmes et des équipements protecteurs de toutes sortes. Cette étude est destinée à faire partie d'un programme TTCP visant à obtenir les données manquantes de toxicité pour des durées d'exposition allant de 10 min à 12 heures.

## Méthodes

Les souris (de souche CD -1 et d'un poids allant de 25 à 30 g) ont été exposées, en groupe de 6, au gaz GB à des niveaux établis pendant une période de 20, 60, 180, 360 ou 720 min. Le compte des décès a été effectué et le  $CL_{t50}$  a été calculé pour chaque durée d'exposition. Les souris qui ont survécu ont été surveillées de 21 à 24 jours; les signes cliniques et leurs poids ont été enregistrés.

## Résultats et discussion

Les toxicités ( $CL_{t50}$ ) GB chez les souris exposées pendant 20, 60, 180, 360 et 720 min ont été estimées être de 430, 538, 899, 1209 et 720  $mg \cdot min/m^3$ , respectivement. En termes de  $CL_{50}$ , les valeurs de toxicité seraient de 21.5, 9.0, 4.9, 3.4 et 3.1  $mg/m^3$ , respectivement. Les valeurs pour les 20 et les 60 min étaient comparables aux valeurs prédites par les modèles de charge toxique développés antérieurement pour des durées d'exposition allant de 10 sec à 30 min. Les valeurs de létalité pour des durées plus longues d'exposition divergent du modèle de charge toxique.

Alors que la durée d'exposition augmentait, la séquence des signes cliniques - salivation excessive (ptyalisme), tremblements, convulsions, paralysie flasque et la mort, sont restés les mêmes pour toutes les durées d'exposition mais l'apparition et le développement des signes cliniques se sont prolongés. On n'a pas observé de cachexie prolongée chez les survivants bien qu'il y ait eu des pertes de poids significantes, immédiatement après l'exposition. Il a fallu 4 à 8 jours pour que les animaux se rétablissent de cette attaque et certains n'avaient toujours pas récupéré leur poids après 21 jours bien que ces souris continuaient à se développer et apparaissaient normales.

La relation entre la toxicité et les durées plus longues d'exposition n'est pas simple. Elle diffère des modèles de charge toxique montrés auparavant pour des durées d'exposition < 30 min. Les valeurs obtenues indiquent une diminution relative en toxicité quand les durées d'exposition augmentent. Les données semblent indiquer une tendance vers une valeur  $CL_{50}$  unique proche de  $3 \text{ mg/m}^3$  alors que la durée d'exposition a été augmentée à 12 heures. Ceci suggère qu'il existerait un seuil pratique des effets mortels pour les durées d'exposition allant jusqu'à 12 heures alors que la courbe toxicité/exposition et durée/effet approche une valeur constante. Cependant, comme les effets non mortels n'ont pas été étudiés, il est possible que d'autres effets physiologiques tels que le myosis, suivent un modèle différent de réactions quand la durée de l'exposition augmente.

## Conclusions

Les valeurs de toxicité par inhalation (létalité) pour les souris ont été estimées pour des durées d'exposition au GB allant de 20 à 720 min

La toxicité apparente par inhalation de GB pour des durées d'exposition allant de 60 à 720 min ne suivent pas la règle d'Haber<sup>2</sup> ni le modèle de toxicité qui décrit les effets pour des durées allant de 0.17 à 30 min.

Les valeurs de toxicité létale de  $CL_{50}$  n'ont changé qu'en petites quantités alors que la durée d'exposition s'étendait de 360 à 720 min. Si cet effet est prouvé similaire chez les autres espèces, ceci peut indiquer que, pour beaucoup d'activités militaires, il existe une valeur de seuil de létalité pour le GB.

Bide, R.W. and Risk, D.J. (2002). GB toxicity in mice exposed for 20 to 720 min. (DRDC Suffield TR 2002-031). Defence R&D Canada – Suffield.

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<sup>2</sup> La règle d'Haber établit que la  $CL_{50} = CT$  quand C représente la concentration et T la durée d'exposition.

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GB was synthesized and purified by Dr. P. Lecavalier of the Chemical Biological Defence Section, DRDC Suffield.

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## Introduction

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When the nerve agents were first studied shortly after WWII, the experiments done to define toxicity were designed to describe the offensive utility of and the necessary defensive measures against these new agents. In these early experiments [1, 2], many exposure times were short (1 to 30 sec) to demonstrate the lethality resulting from a massive attack and incapacitation from a single breath and very little data was generated for exposures >10 min. When the literature concerning nerve agents was re-examined in preparation for the 1991 Gulf War, one of the main data gaps identified was the absence of data concerning the toxicity of the nerve agents at exposure times greater than 15 min [1, 2, 3, 4]. Information concerning the toxicity of CW agents at these longer exposure times are critical for;

1. personnel functioning in collective protection when contamination levels can be low and exposures of many hours are encountered,
2. flight crew waiting for action in areas with low level contamination,
3. operational aircrew when previously contaminated materiel is confined for long flights in cargo bays,
4. design of detection equipment, monitors, alarm systems and protective equipment of all kinds,
5. hospital staff who must work with contaminated victims (as clearly demonstrated during a recent terrorist incident in Tokyo [5, 6, 7, 8]) and
6. all personnel that have been attacked, are dressed in Individual Protective Equipment, and need to know when, and if, it is safe to take off these cumbersome garments.

As the first step in Remediation of these data gaps, The Technical Cooperation Program (TTCP) formed an Action Group (AG-45) in 1998 to critically assess the situation and begin, collaboratively when possible, the research effort to provide longer term toxicologic values for the nerve agents. The AG was upgraded to a Technical Panel (TP-12) in 1999 and charged with the task of remediation of the knowledge/data gaps for all chemical warfare agents. As part of the Canadian effort in support of TP-12, estimations of the toxicity of GB for 20 min to 12 hr exposures were undertaken in mice. The resulting data, presented here, are compared to the historical information collected at shorter exposure times [1, 2].

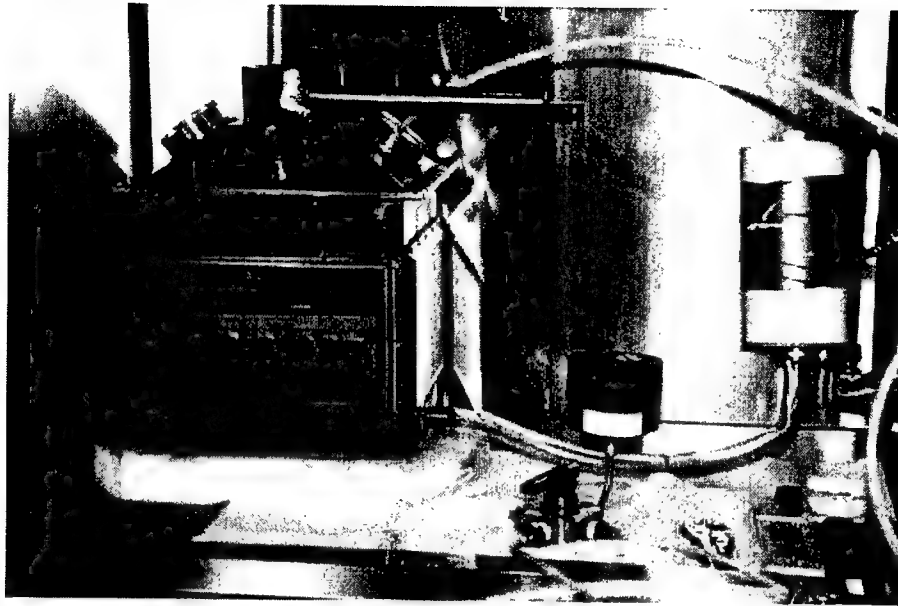
## Materials and methods

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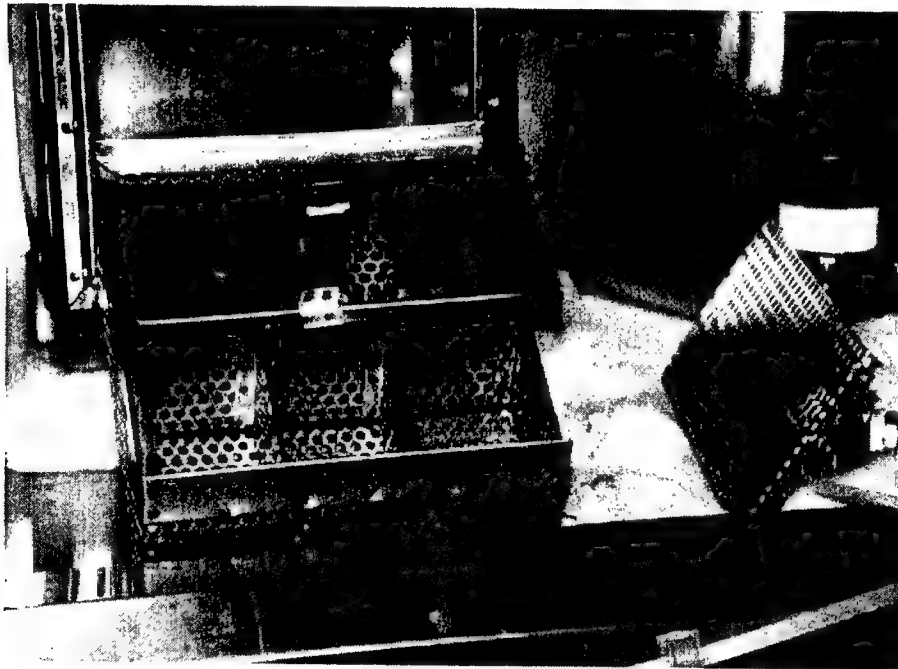
### Chemicals

GB (isopropyl methylphosphonofluoridate, CAS # 107-44-8 or 50642-23-4, MW 140) was synthesized, *de novo*, at DRDC Suffield and was freshly distilled shortly before use. The purity (>98%) was regularly tested by NMR during the experiments. The <sup>31</sup>P- and <sup>1</sup>H-NMR spectra are in Annex A.

A



B



**Figure 1.** The exposure chamber (A) and the animal holder (B). Exposure gas enters by the horizontal tube at the top of the chamber and exhausts at the bottom through a carbon filter. The mask filter is part of the calibration loop. The tube leading from the angled face on the top right of the chamber leads from the centre of the chamber to the MIRAN which monitors the exposure concentration. The animal holder (B) is shown with one closure removed.

## Animals

The mice used in this study were male outbred CD-1<sup>®</sup> strain purchased from Charles River Canada, St. Constant, PQ. The mice were acclimatized for at least 7 days in the Vivarium at DRDC Suffield before use.

## Air supply

Particle and chemical free air was supplied by a breathing air system consisting of a water sealed compressor (SIHI model KHPE 35504 SIHI-Halberg International Co., Ludwigshafen/Rhein, West Germany) fitted with HEPA filters on the air intake, a water separator and air dryer immediately following the compressor and then oil and particle filters in the compressed air line. The resulting dry, particle and chemical free air was delivered at 60 lb/in<sup>2</sup> (413 KNeutons/m<sup>2</sup>) to the inhalation exposure system.

## Exposure system

Animal exposures were carried out in a dynamic exposure system consisting of (in progressive order) a Tylan flow controller (Model RO-28 with FC-262-V; Tylan General, Torrance CA), injection port created from a stainless steel Swagelok<sup>®</sup> T-union with a septum fitted to one arm of the "T", a MIRAN 1A Analyser (Model CVF; General Purpose Gas Analyzer; Foxboro Analytical, South Norwalk, CT), a custom made Stainless Steel and Glass exposure chamber of 31.75 L interior volume and 900 cm<sup>2</sup> interior cross section (Fig. 1a; designed by BioResearch Inc., Senneville, PQ. under contract to DRDC Suffield). The exhaust was passed through an M18 military tank filter (US (NATO) # 4240-00-828-3952). All piping connections were made with 3/8" ID (9.53 mm) polypropylene tubing. A gas sample was drawn continuously at 10 L/min from immediately above the animal holder into a second MIRAN Analyzer for estimation of the exposure concentration.

The six compartment animal holder, when placed in the chamber, rested upon a wire mesh support with a perimeter barrier designed to ensure that all of the exposure gas passed through the animal holder.

During exposures, the trial chemical (neat GB) was introduced to the input air at the injection port from a microliter Hamilton Gastight syringe (Hamilton Co., Reno, NE) driven by a Model 22 Syringe Pump (Harvard Apparatus, Inc., Holliston, MA).

## Exposure concentrations

Both MIRAN Analyzers were running continuously during all exposures. The exposure concentration was monitored only by the MIRAN sampling from the exposure chamber.

The MIRAN Analysers were calibrated daily. For calibration, the two MIRAN Analyzers, a Metal Bellows pump (15 L/min), the injection port and an SS Swagelok valve were arranged in a closed loop. The valve allowed either a dynamic flow through the Analyzers (purge setting) or a closed recirculating loop (calibration). To calibrate, a series of measured amounts

of GB were introduced into the closed loop. After each injection, the gas in the loop was allowed to equilibrate and, then, the loop was purged. To simplify subsequent calculations, the area under the tracing for one chart division at equilibrium was used for the calibration value for each peak. To estimate the total exposure, the recorder tracings were divided into segments and the area under the segment measured. The average area for one recorder chart division in each segment was calibrated into  $\text{mg} \cdot \text{min}/\text{m}^3$  and multiplied by time represented by the number of chart divisions in the segment. The values for the segments were then summed. For each experiment, the chart speed was the same for both calibration and exposure.

## Animal exposures

In operation, the air flow through the system was regulated at 20 L/min. The animals, in pre-weighed groups of 6, were placed in the animal holder in random order and the holder placed into the chamber. The system was sealed and air flow started. After the instruments had stabilised and baseline was established, the syringe pump was started and the exposures were timed from the first detection of chemical by the MIRAN sampling from inside the chamber. After the prescribed exposure time, the syringe pump was stopped and the syringe removed from the injection port. The chamber was opened when the MIRAN sampling from inside the chamber indicated that the atmosphere in the chamber was clear of agent (usually 8 min after injection stopped). The animals were removed, placed in caging for observation and then examined regularly for the remainder of the working day. The groups were examined and weighed daily to the end of that working week and then twice weekly until 21-24 days after exposure.

Because of the possibility of agent ingestion from contaminated food or water, neither were provided during the animal exposures.

One group of mice were kept as controls. These were only handled for marking, weighing and examinations. For each exposure time, a group of 6 mice were mock exposed in the chamber - no GB - and then examined in the same manner as the exposed groups to demonstrate the effects of stress from the exposure process. The results of all test groups were compared to both of these controls. For brevity, the data for the control group are given only in Table 1.

## Calculations and statistics

The exposure for each experiment was calculated by integrating the recorder trace produced by the MIRAN sampling from the exposure chamber and then calculating the concentration using the daily calibration of the system. In each experiment, the  $t_{99}$  for that exposure was measured on the MIRAN fed from the centre of the chamber.

The data generated (exposure concentrations and times, body weights, etc.) were assembled into tables using FRAMEWORK VI<sup>®</sup> software<sup>3</sup> and programs written in this laboratory. Various summary tables were then prepared according to the needs of the experimental work.

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<sup>3</sup> FRAMEWORK<sup>®</sup>, originally written by Ashton Tate, was not supported when Borlan purchased that company. FRAMEWORK<sup>®</sup> VI, the latest version, which runs under Windows 95, is available from Selections & Functions, Inc., Box 505, Scituate, MA 02066.

Calculation of the LCt<sub>16</sub>, LCt<sub>50</sub> and LCt<sub>84</sub> values were done using the established methods of Litchfield and Wilcoxon [9] using a commercial program [10]. Mean body weights were compared to the initial body weights, first using analysis of variance (F-test) and then Duncan's Multiple Range test. The body weights on each day were compared to the corresponding control using two-tailed Student's *t* tests [11].

## Results

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The toxic responses seen in the exposed mice were those consistent with organophosphate poisoning such as excess salivation (ptyalism), piloerection, tremors, convulsions and flaccid paralysis preceding death. With the few exceptions noted below, the affected animals exhibited these clinical signs in sequence at progressive times during the exposures and the casualties died either within the exposure chamber or very shortly after they were removed from the chamber at the end of the exposures. No abnormal clinical signs were observed and post-mortem and histologic examinations did not reveal any lesions or effects that were not directly attributable to the organophosphate poisoning.

### 20 min exposures

Seven groups of mice were exposed to GB concentrations between 18.6 and 24.2 mg/m<sup>3</sup> or 372 and 483 mg.min/m<sup>3</sup> GB (Table 1). The *t*<sub>99</sub> values<sup>4</sup> recorded for these exposures were 6.6 min with a standard error (SE) of 0.17. For these exposures one chart division represented 0.2 min. For one exposure, the results of 10 intervals were averaged to provide a measure of the precision of the GB exposure (482 ± 47 mg.min/m<sup>3</sup>).

The calculated values for toxicity and 95% confidence limits were 337 (319:355), 430 (387:478) and 549 (520:579) mg.min/m<sup>3</sup>, for LCt<sub>16</sub>, LCt<sub>50</sub> and LCt<sub>84</sub>, respectively. The PROBIT equation was

$$Y = 9.3902 \cdot \log_{10}(Ct) - 19.7278.$$

Times to death were all < 30 min from the start of exposure.

The control mice and the mock exposed had essentially the same pattern of body weight changes, indicating that the mock exposure for 20 min had little effect.

Body weight loss was recorded in all survivors on day 1. On day 2, only the survivor of the first 468 mg.min/m<sup>3</sup> exposure (marked with an asterisk in Table 1) had a body weight greater than the starting weight. The values for the remaining groups were significantly different from both control and mock exposed mice. Body weight gains resumed after day 2 and continued until the end of the observation period when the average body weights of all survivors were similar to those of the control group.

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<sup>4</sup> *t*<sub>99</sub> refers to the time required for the exposure atmosphere to attain 99% of the operational value.

**Table 1. Toxicity of GB to Mice; 20 min exposure**

Exposure (mg.min) m <sup>3</sup> )	Mortality	Body weight (g)	Per cent change in body weight by day							
			1	2	6	9	13	16	20	23
<i>Control</i>	0	25.9 ±0.82	4.6 <sup>b</sup> ±1.7	6.7 <sup>b</sup> ±3.4	15 <sup>a</sup> ±2.7	20 <sup>a</sup> ±2.9	20 <sup>a</sup> ±3.3	24 <sup>a</sup> ±3.2	27 <sup>a</sup> ±3.7	28 <sup>a</sup> ±5.0
<i>Mock exposed</i>		26.5 ±1.5	-2.4 ±3.6	2.6 ±2.4	11 <sup>b</sup> 8.7	20 <sup>a</sup> ±10	24 <sup>a</sup> ±15	25 <sup>a</sup> ±11	27 <sup>a</sup> ±15	32 <sup>a</sup> ±15
<i>GB trials</i>										
483	5/6	25.7 ±0.77	-12	-9.9	8.2	15	23	25	27	32
468 <sup>*</sup>	5/6	26.2 ±1.0	-0.70	0.12	12	18	26	26	28	31
468	3/6	25.6 ±1.2	-6.7 <sup>cf</sup> ±5.4	-4.8 <sup>ce</sup> ±3.3	9.0 <sup>ad</sup> ±5.5	12 <sup>bd</sup> ±7.7	16 <sup>a</sup> ±8.5	17 <sup>ad</sup> ±5.8	22 <sup>a</sup> ±5.8	25 <sup>a</sup> ±4.4
451	3/5	25.2 ±1.1	-1.96	0.03	15.0	20	25	26	33	33
372	3/6	26.2 ±0.49	-7.1 <sup>ace</sup> ±2.6	-4.5 <sup>bce</sup> ±3.2	9.5 <sup>ac</sup> ±3.2	14 <sup>ad</sup> ±5.3	21 <sup>a</sup> ±5.1	24 <sup>a</sup> ±3.6	29 <sup>a</sup> ±3.8	32 <sup>af</sup> ±4.2
372	2/6	26.4 ±1.7	-10.5 <sup>bce</sup> ±3.6	-6.9 <sup>ce</sup> ±3.7	7.9 <sup>c</sup> ±3.1	14 <sup>ac</sup> ±4.0	21 <sup>a</sup> ±4.4	23 <sup>a</sup> ±3.6	27 <sup>a</sup> ±4.4	30 <sup>a</sup> ±5.1
410	0/6	25.8 ±0.67	-6.8 <sup>bce</sup> ±3.9	-3.6 <sup>ce</sup> ±3.3	7.5 <sup>ac</sup> ±1.9	12 <sup>ac</sup> ±2.2	17 <sup>a</sup> ±3.1	19 <sup>ae</sup> ±1.6	22 <sup>ad</sup> ±3.8	27 <sup>a</sup> ±4.8

Values are mean ± standard deviation. Control mice were not cycled through the chamber system.

Mock exposed animals were 60 min in the exposure chamber without GB exposure

<sup>a</sup> Significantly different to Day 0;  $P < 0.01$

<sup>b</sup> Significantly different to Day 0;  $P < 0.05$ .

<sup>c</sup> Significantly different to control;  $P < 0.05$ .

<sup>d</sup> Significantly different to control;  $P < 0.01$ .

<sup>e</sup> Significantly different to mock exposed;  $P < 0.01$ .

<sup>f</sup> Significantly different to mock exposed;  $P < 0.05$ .

**Table 2. Toxicity of GB to Mice; 60 min exposure**

Exposure (mg.min m <sup>3</sup> )	Mortality	Body weight (g)	Per cent change in body weight by day								
			1	2	3	6	9	13	16	20	23
<i>Mock exposed</i>		25.9 ±0.96	3.0 ±1.7	5.6 <sup>a</sup> ±2.8	8.7 <sup>a</sup> ±2.7	13 <sup>a</sup> ±2.8	15 <sup>a</sup> ±2.6	20 <sup>a</sup> ±3.8	27 <sup>a</sup> ±8	28 <sup>a</sup> ±8	35 <sup>a</sup> ±11
<i>GB trials</i>											
689	6/6	24.9 ±1.6									
557	5/6	23.8 ±1.5	-6.4	-5.0	0.09	11	12	15	21	24	25
550	3/6	24.6 ±0.93	-16 <sup>ace</sup> ±4.8	-15 <sup>ace</sup> ±7.8	-6.5 <sup>ce</sup> ±8.1	3.4 <sup>dfb</sup> ±7.0	7.7 <sup>adf</sup> ±6.2	16 <sup>d</sup> ±5.7	21 <sup>d</sup> ±5.9	25 <sup>d</sup> ±6.9	25 <sup>adf</sup> ±5.4
518	1/6	24.0 ±1.7	-3.4 <sup>d</sup> ±6.3	0.30 <sup>ce</sup> ±2.6	4.7 <sup>f</sup> ±2.4	12 <sup>cc</sup> ±2.1	14 <sup>ac</sup> ±2.6	22 <sup>a</sup> 3.5	26 <sup>a</sup> ±4.4	31 <sup>a</sup> ±4.6	31 <sup>a</sup> ±5.0
412	0/5	27.8 ±1.1	-0.58 <sup>d</sup> ±4.1	0.47 <sup>df</sup> ±5.0	4.1 <sup>af</sup> ±3.7	7.7 <sup>acf</sup> ±4.2	9.4 <sup>ac</sup> ±6.1	13 <sup>adf</sup> ±7.3	17 <sup>adf</sup> ±7.5	19 <sup>a</sup> ±9.1	22 <sup>adf</sup> ±9.2

Values are mean ± standard deviation. Data for control animals are given in Table 1.

Mock exposed animals were 60 min in the exposure chamber without GB exposure

<sup>a</sup> Significantly different to Day 0;  $P < 0.01$

<sup>b</sup> Significantly different to Day 0;  $P < 0.05$ .

<sup>c</sup> Significantly different to control;  $P < 0.05$ .

<sup>d</sup> Significantly different to control;  $P < 0.01$ .

<sup>e</sup> Significantly different to mock exposed;  $P < 0.01$ .

<sup>f</sup> Significantly different to mock exposed;  $P < 0.05$ .

## 60 min exposures

Five groups of mice were exposed to GB concentrations between 6.9 and 11.5 mg/m<sup>3</sup> or 412 and 689 mg.min/m<sup>3</sup> GB (Table 2). The  $t_{99}$  values recorded for these exposures were 5.0 min with an SE of 0.38. For these exposures, one chart division was 0.8 min. For one exposure, the results of 10 intervals were averaged to provide a measure of the precision of the GB exposure (410 ± 13 mg.min/m<sup>3</sup>).

The calculated toxicity values - LC<sub>16</sub>, LC<sub>50</sub> and LC<sub>84</sub> with 95% confidence limits - for 60 min exposures were 491 (490:492), 538 (507:571) and 589 (588:591) mg.min/m<sup>3</sup>, respectively. The PROBIT equation was

$$Y = 25.2304 \cdot \log_{10}(Cr) - 63.8993.$$

Clinical signs of toxicity were not seen until the last 10 min of the exposure period. The times for each stage of the toxic response were not timed but the impression was that these were extended in comparison to the 20 min exposures. Times to death were close to the exposure time and no deaths occurred after one hr post exposure.

**Table 3. Toxicity of GB to Mice; 180 min exposure**

Exposure (mg.min m <sup>3</sup> )	Mortality	Body weight (g)	Per cent change in body weight by day								
			1	2	3	4	6	8	14/15	16/18	21
Mock exposed		28.1 ±0.96	-3.0 <sup>dc</sup> ±2.4	-0.18 <sup>dc</sup> ±2.7	1.3 <sup>c</sup> ±3.3	0.66 ±3.2	5.0 <sup>c</sup> ±3.7	3.5 <sup>c</sup> ±4.0	12 <sup>ac</sup> ±3.2	14 <sup>ac</sup> ±3.3	15 <sup>ac</sup> ±3.6
GB trials											
1100	6/6	24.6 ±1.9									
1017	4/6	25.1 ±1.4	-10.6	-4.3			5.2	12	17	18	24
810	2/6	25.7 ±2.2	-13 <sup>ac</sup> ±4.5	-9.6 <sup>bce</sup> ±3.3	-2.8 <sup>c</sup> ±3.9		4.6 <sup>c</sup> ±3.8	12 <sup>ce</sup> ±4.4	19 <sup>bf</sup> ±5.3	22 <sup>ae</sup> ±4.2	27 <sup>ae</sup> ±5.0
788	2/6	26.7 ±0.7	-10.0 <sup>ace</sup> ±2.5			-0.85 ±1.8	2.4 <sup>c</sup> ±2.2	7.2 <sup>ac</sup> ±2.5	13 <sup>ac</sup> ±3.2	16 <sup>ac</sup> ±3.3	17 <sup>ac</sup> ±4.5
725	2/6	26.2 ±2.6	-7.4 <sup>bce</sup> ±2.1			-1.6 ±2.7	1.5 <sup>c</sup> ±4.1	5.6 <sup>bc</sup> ±5.2	14 <sup>b</sup> ±8.7	18 <sup>a</sup> ±9.7	17 <sup>ad</sup> ±9.1
772	0/6	25.9 ±1.2	-9.2 <sup>bc</sup> ±7.5	-7.4 <sup>bce</sup> ±5.2	-1.3 <sup>c</sup> ±5.5		4.4 <sup>c</sup> ±3.0	11 <sup>ace</sup> ±2.9	17 <sup>af</sup> ±3.7	20 <sup>ade</sup> ±3.5	24 <sup>ae</sup> ±5.1

Values are given as mean ± standard deviation. Control data are given in Table 1.

Mock exposed animals were in the exposure chamber for 180 min without GB.

<sup>a</sup> Significantly different to Day 0;  $P < 0.01$ .

<sup>b</sup> Significantly different to Day 0;  $P < 0.05$ .

<sup>c</sup> Significantly different to control;  $P < 0.01$ .

<sup>d</sup> Significantly different to control;  $P < 0.05$ .

<sup>e</sup> Significantly different to mock exposed;  $P < 0.01$ .

<sup>f</sup> Significantly different to mock exposed;  $P < 0.05$ .

The mock exposed group did not show significant body weight changes when compared to the control group. After 23 days, the average body weight of the mock exposed mice was higher than the control but the difference was not statistically significant because of the high standard deviations involved.

For the GB exposed mice, significant body weight losses were recorded (Table 2) in all survivors on day 1. The differences were significant compared to day 0, control and mock exposed groups. Body weight gains resumed on day 2 in the groups exposed to 412 and 518 mg.min/m<sup>3</sup> and continued until the end of the 23 day observation period when these mice reached the body weights of the control group. In the group exposed to 550 mg.min/m<sup>3</sup>, the initial recovery was much slower and body weights below the starting values were recorded for 3 days. At day 23 the body weights were equal to those of the control and mock exposed groups.

## 180 min exposures

Six groups of mice were exposed to GB concentrations between 4.29 and 6.11 mg/m<sup>3</sup> or 772 and 1100 mg.min/m<sup>3</sup> (Table 3). The  $t_{99}$  values for these exposures were 5.1 min with an SE of 0.17. For these exposures, one chart division was 1.2 min. For one exposure, the results of 10 intervals were averaged to provide a measure of the precision of the GB exposure (772 ± 31 mg.min/m<sup>3</sup>).

The calculated toxicity values and 95% confidence limits - LC<sub>t16</sub>, LC<sub>t50</sub> and LC<sub>t84</sub> - for 180 min exposures were 688 (678:697), 899 (795:1018) and 1176 (1160:1194) mg.min/m<sup>3</sup>, respectively. The PROBIT equation was

$$Y = 8.5237 \cdot \log_{10}(Ct) - 20.1800.$$

The first onset of clinical signs came after 140 min. Times to death were close to, but less than, the exposure time as all casualties died before the end of the exposure period.

The mock exposed group showed body weight loss on days 1 and 2. The body weights were not significantly different from day 0. However, these values were significantly below the control values and the body weights of this group remained significantly below the control for the 21 days of the experiment. The responses of individual mice were varied as indicated by the relatively higher standard deviations recorded.

For GB exposed mice, significant body weight loss (Table 3) was recorded in all survivors on day 1 compared to day 0, control and mock exposed groups. Although recovery had started by day 2, body weights above those on day 0 were not recorded until day 6. On day 21, all animals were growing well and some had gained enough that the groups were not significantly different from the controls.

## 360 min exposures

Six groups of mice were exposed to GB concentrations between 2.7 and 4.1 mg/m<sup>3</sup> or 969 and 1493 mg.min/m<sup>3</sup> (Table 4). The  $t_{99}$  values for these exposures were 5.5 min with an SE of 0.30. For these exposures, one chart division was 1.53 min. For one exposure, the results of 10 intervals were averaged to provide a measure of the precision of the GB exposure (1228 ± 33 mg.min/m<sup>3</sup>).

The calculated toxicity values with 95% confidence limits - LC<sub>t16</sub>, LC<sub>t50</sub> and LC<sub>t84</sub> - for 360 min exposures were 931 (838:1033), 1209 (795:1018) and 1571 (1415:1744) mg.min/m<sup>3</sup>, respectively. The PROBIT equation was

$$Y = 8.7456 \cdot \log_{10}(Ct) - 21.9586.$$

In the group of mock exposed mice, initial significant body weight loss (compared to control) was quickly reversed and growth continued for 23 days.

Clinical signs of toxicity were first evident about 300 min and slowly became more pronounced as the exposure continued. With one exception, the casualties died within a short time of the end of the exposure period. The one mouse that survived the 1474 mg.min/m<sup>3</sup> exposure did not thrive and was essentially the same body weight after 23 days. In the groups exposed to 969, 1364 and 1386, there was significant body weight loss on days 1 - 3

**Table 4. Toxicity of GB to Mice; 360 min exposure**

Exposure (mg.min /m³)	Mortality	Body weight (g)	Per cent change in body weight by day								
			1	2	3	4	6/7	10	14	19	23
Mock exposed		27.3 ±1.4	-1.8 <sup>c</sup> ±1.5	-0.28 <sup>c</sup> ±1.5	1.7 <sup>c</sup> ±1.0	3.5 ±2.1	7.1 <sup>ad</sup> ±2.5	14 <sup>a</sup> ±3.7	19 <sup>a</sup> ±9.7	23 <sup>a</sup> ±5.8	30 <sup>ac</sup> ±5.8
GB trials											
1493	6/6	32.3 ±1.4									
1474	5/6	31.5 ±1.6	-12.4				-8.9	-3.2		0.75	-0.12
1235	6/6	31.9 ±1.5	One animal found dead day 2								
1386	1/6	25.8 ±1.4	-3.9 <sup>cd</sup> ±1.5	-1.4 <sup>c</sup> ±1.8	1.4 <sup>c</sup> ±1.2	3.0 ±2.2	6.9 <sup>bc</sup> ±4.2	12 <sup>ac</sup> ±5.2	15 <sup>a</sup> ±5.7	24 <sup>a</sup> ±7.5	21 <sup>ac</sup> ±7.4
1364	3/6	26.0 ±1.4	-12 <sup>ce</sup> ±7.9	-14 <sup>cd</sup> ±14	-7.0 <sup>d</sup> ±12	-2.2 ±9.6	-0.94 <sup>c</sup> ±10.7	6.5 <sup>c</sup> ±10.0	10.5 <sup>bd</sup> ±10.5	17 <sup>ad</sup> ±7.8	19 <sup>ac</sup> ±9.1
969	1/6	32.0 ±2.3	-2.2 <sup>c</sup> ±2.6	-1.9 <sup>c</sup> ±2.4			2.3 <sup>c</sup> ±4.8	6.4 <sup>bc</sup> ±6.1	8.3 <sup>ace</sup> ±5.8	112 <sup>ac</sup> ±5.4	13 <sup>acf</sup> ±5.7

Values are given as mean ± standard deviation. Control data is in Table 1.

Mock exposed animals were 360 min in the exposure chamber with nom GB.

<sup>a</sup> Significantly different to Day 0;  $P < 0.01$ .

<sup>b</sup> Significantly different to Day 0;  $P < 0.05$ .

<sup>c</sup> Significantly different to control;  $P < 0.01$ .

<sup>d</sup> Significantly different to control;  $P < 0.05$ .

<sup>e</sup> Significantly different to mock exposed;  $P < 0.01$ .

<sup>f</sup> Significantly different to mock exposed;  $P < 0.05$ .

compared to the control and, in those exposed to 1364 mg.min/m<sup>3</sup>, to the mock exposed group as well. This latter group of three surviving mice contained two mice that were only slightly affected and one that was very severely affected - hence the large standard deviations recorded.

## 720 min exposures

Seven groups of mice were exposed to GB concentrations between 1.9 and 3.2 mg/m<sup>3</sup> or 1366 and 2303 mg.min/m<sup>3</sup> (Table 5). The  $t_{99}$  values recorded for these exposures were 5.5 min with an SE of 0.15. For these exposures, one chart division represented 2.0 min. For one exposure, 10 intervals were averaged to provide a measure of the precision of the GB exposure (1617 ± 41 mg.min/m<sup>3</sup>).

The PROBIT equation calculated using the data for all seven exposure concentrations was

$$Y = 15.7214 \cdot \log_{10}(Ct) - 47.6690$$

**Table 5. Toxicity of GB to Mice; 720 min exposure**

Exposure (mg.min /m <sup>3</sup> )	Mort- ality	Body weight (g)	Per cent change in body weight by day									
			1	2	4	5	6	7	8	13	17	20
Mock exposed		27.1 ±1.7	-4.0 <sup>c</sup> ±4.7	-2.4 <sup>c</sup> ±2.8	-1.0 ±1.6	1.1 ±4.2	3.2 <sup>c</sup> ±3.9	4.5 <sup>c</sup> ±4.0	3.6 <sup>bc</sup> ±3.7	8.9 <sup>bc</sup> ±3.2	13 <sup>ac</sup> ±3.9	13 <sup>ac</sup> ±3.9
<b>GB trials</b>												
2303	6/6	28.6 ±1.1										
2313	5/6	26.3 ±1.4	-20	-17	-4.6	-4.6	-2.0	0.07	3.2	-	17	21
2081	2/6	30.1 ±1.2	-10.3 <sup>acd</sup> ±3.6	-7.2 <sup>cd</sup> ±3.4	-3.4 <sup>f</sup> ±1.6	-2.6 <sup>e</sup> ±1.6	-1.8 <sup>ce</sup> ±1.4	-0.8 <sup>cd</sup> ±1.2	-	7.9 <sup>ac</sup> ±4.6	11 <sup>ac</sup> ±2.4	13 <sup>ac</sup> ±3.4
1625	0/6	25.7 ±1.8	-10.0 <sup>bcd</sup> ±3.1	-6.8 <sup>cd</sup> ±2.4	-1.1 <sup>e</sup> ±2.5	0.17 ±2.2	2.2 <sup>c</sup> ±2.4	3.7 <sup>c</sup> ±2.4	4.8 <sup>c</sup> ±2.6	11 <sup>bc</sup> ±3.3	-	16 <sup>ac</sup> ±3.5
2200	1/6	29.0 ±2.6	-9.9 <sup>cd</sup> ±2.6	-4.7 <sup>c</sup> ±3.6	-	-	-	5.1 <sup>c</sup> ±2.6	-	7.6 <sup>bc</sup> ±3.3	10.1 <sup>ac</sup> ±3.7	12 <sup>ac</sup> ±4.3
2096	0/6	29.2 ±1.8	-10.7 <sup>acd</sup> ±2.9	-6.8 <sup>bcd</sup> ±2.4	-2.0 <sup>c</sup> ±2.5	-1.1 <sup>f</sup> ±2.8	0.50 <sup>f</sup> ±2.3	1.5 <sup>c</sup> ±2.4	2.9 <sup>c</sup> ±3.1	-	9.4 <sup>ac</sup> ±3.3	13 <sup>ac</sup> ±3.9
1366	0/6	25.6 ±2.0	-7.2 <sup>c</sup> ±3.4	-3.6 <sup>c</sup> ±2.4	1.4 ±2.8	6.2 ±3.5	7.7 <sup>c</sup> ±4.1	8.4 <sup>bcd</sup> ±4.2	9.8 <sup>bc</sup> ±4.7	17 <sup>af</sup> ±7.5	-	25 <sup>ae</sup> ±5.1

Values are given as mean ± standard deviation. Control data are in Table 1.

Mock exposed mice were 720 min in the exposure chamber without GB.

<sup>a</sup> Significantly different to Day 0;  $P < 0.01$ . <sup>b</sup> Significantly different to Day 0;  $P < 0.05$ .

<sup>c</sup> Significantly different to control;  $P < 0.01$ . <sup>d</sup> Significantly different to control;  $P < 0.05$ .

<sup>e</sup> Significantly different to mock exposed;  $P < 0.01$ .

<sup>f</sup> Significantly different to mock exposed;  $P < 0.05$ .

and the indicated values for  $LC_{t_{16}}$ ,  $LC_{t_{50}}$  and  $LC_{t_{84}}$  were 1936, 2239 and 2591 mg.min/m<sup>3</sup>, respectively. However, the lowest exposure (1366 mg.min/m<sup>3</sup>) was considerably below the 2000 mg.min/m<sup>3</sup> that appeared to be the low value that caused lethal effect. Further, the program used to calculate the PROBIT lines [9, 10] recommends two zero response values only. When the data for the group exposed to 1366 mg.min/m<sup>3</sup> was removed from the PROBIT calculation, the equation became

$$Y = 22.8679 \cdot \log_{10}(Ct) - 71.4991$$

The potency ratio between these two equations indicated that the results were not significantly different so the latter PROBIT equation was used. The  $LC_{t_{16}}$ ,  $LC_{t_{50}}$  and  $LC_{t_{84}}$  values with 95% confidence limits were calculated to be 2003 (1999:2007), 2214 (2103:2331) and 2447 (2442:2453) mg.min/m<sup>3</sup>, respectively. From both calculations, the resulting toxicity curves were extremely steep. The difference between  $LC_{t_{16}}$  and  $LC_{t_{84}}$  was 444 in 2447 or 18 per cent.

Clinical signs of poisoning were not evident until near the end of the exposure period. Casualties died either in the last 10% of the exposure or in the immediate post exposure period. The surviving mice all showed some clinical signs as they were removed from the exposure chamber. In the survivors, these signs rapidly cleared.

The 720 min mock exposure caused a significant decrease in body weights of the mice on the first day following treatment. Body weight was recovering on day 2 but growth was slower than in the control. Body weight was not significantly increased over day 0 until day 6 and the mice did not achieve parity with the control by day 21.

Body weight was significantly reduced on day one in all GB exposed groups when compared to the control. The average weight loss was 10 per cent when compared to day 0 but this value was statistically significant for only three of the five groups because of the higher standard deviations recorded. In the groups exposed to 2096 - 2313 mg.min/m<sup>3</sup> GB, the weight loss was also significantly greater than in the mock exposed mice. The change in the group exposed to 1366 mg.min/m<sup>3</sup> GB was numerically greater than the mock exposed but the larger standard deviations recorded precluded statistical significance. Recovery had begun in most cases by day 2, but was very much slower than in the control and mock exposure groups so that significant differences to the control were recorded until day 3 and to the mock exposed group until day 4. The body weights in the group exposed to 2081 mg.min/m<sup>3</sup> were still significantly lower than the mock exposed on day 7. Growth in the survivors was at least equal to that in the mock exposed mice by day 21 but all body weights were significantly lower than the control.

## Discussion

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The toxicity values obtained in these experiments (Table 6) should be consistent within the study as all were generated in a similar system, using similar animals (all male, one outbred strain, close to same size and age) and using GB of known purity from the same source. The 95% confidence limits of the values obtained were a similar proportion (0.74 - 0.94) of the toxicity values indicating that the precision of the toxicity estimates were similar in these experimental groups.

As the exposure time increases, the lethal concentration (LC<sub>50</sub>) appears to be approaching a threshold value about 3 mg/m<sup>3</sup>. Unfortunately, the PROBIT slope and intercept values for the toxicity calculations did not follow a similar pattern to the LC<sub>50</sub> values (Table 6) so that a confident or rigorous prediction of the LC<sub>05</sub> or LC<sub>01</sub> values was not possible. However, as a first approximation, a value of 8 may be used for the PROBIT slope. This is the rounded down value of the shallowest experimental PROBIT slope (8.52; 180 min). The caveats for both the LC<sub>50</sub> and the PROBIT slope are extensive. The data were generated using mice of a single source, strain, sex and body size, fed a uniform diet and known to be essentially healthy and pathogen free as well as having had no recent exposure to toxic materials. This is very different from the situation encountered in either wild mice or a human population [12]. A much lower PROBIT slope would be expected for the population, reflecting the greater variability in the subjects, and, indeed, this was seen in combined data from several mouse experiments [1, 2].

**Table 6. Summary of GB toxicity to mice**

Exposure time (min)	Toxicity			PROBIT slope	Intercept
	LCt <sub>16</sub>	LCt <sub>50</sub>	LCt <sub>84</sub>		
20	337 (319:355)	430 (387:478)	549 (520:579)	9.390	-19.73
60	491 (490:4920)	538 (507:571)	589 (588:591)	25.230	-63.90
180	688 (678:697)	899 (795:1018)	1176 (1160:1194)	8.524	-20.18
360	931 (838:1033)	1209 (941:1554)	1571 (1415:1744)	8.746	-21.96
720	2003 (1999:2007)	2214 (2103:2331)	2447 (2442:2453)	22.867	-71.50
Values are given as LCt <sub>x</sub> and (95% confidence limits)					

The extended exposure periods were clearly stressful to the mice as seen by the progressive body weight changes recorded in the tables. There was little effect compared to the controls after 20 or 60 min mock exposure. With the 180 - 720 min GB exposures, the body weight changes generally were more severe than those recorded for the mock exposures and the lower body weights were recorded for several days. In general, growth was only temporarily affected. However, with the 720 min mock exposures, the mice had lower body weights than the control at the end of the observation period.

The data clearly indicate that there is a general mild systemic effect of GB exposure on all survivors. The majority recover quickly. However, the effects were not uniform and some mice were very severely affected and did not recover readily. In these animals, the more severe effect of GB resulted in a "malaise" and body weight loss that was sustained over several days. One such individual mouse had just regained its original body weight by day 23. These animals were found in groups in which at least 50 per cent of the subjects succumbed. These animals did not appear abnormal or toxic after the observation period - they just failed to recover at the speed achieved by other subjects. Within the observation period, none of the survivors showed debilitating effects that would cause continuing weight loss. However, the observation period in these experiments is relatively short and the results do not preclude the appearance of clinical signs and symptoms after longer periods.

Previously, the GB toxicity data in mice (and other species) was generated with responses to battlefield attacks in mind. The result was that the majority of the effort was directed towards peracute toxicity *ie.* very short (1 to 30 sec) exposures. The aim was to define the effects from a single breath of contaminated air. In the literature data in mice, only one historical study [13] of the 10 identified [1] contained data for exposures >10 min. The relationship between toxicity, exposure time and lethal concentration (LCt<sub>50</sub>) did not fit "Haber's Rule" (the LCt<sub>50</sub> did not vary directly as the product of concentration and exposure time *ie.* LCt<sub>50</sub> ≠ Ct). When

the historical data was examined [1, 2], it was clear that an empirical, allometric relationship could be defined to relate exposure time and toxicity over the exposure range of 10 sec to 30 min. This allometric relationship or "toxic load" model was described by the equation  $Y = a_0 + a_1 \text{Log}_{10} C^n T$ ; where  $n$  for the mouse population was calculated to be as 1.321 [1].

Assuming that the same relationship would continue to 720 min, toxicity values were predicted for these extended exposure times (Table 7). All predicted  $\text{LC}_{50}$  values are lower than the experimental numbers and, as exposure time increased, the separation increased further in a non-linear fashion. At 720 min exposure, the experimental value for  $\text{LC}_{50}$  was more than double the predicted value. Similarly, the  $\text{LC}_{50}$  values separated as the exposure time increased. As noted previously, the  $\text{LC}_{50}$  value appeared to be approaching a value of  $3 \text{ mg/m}^3$  as the exposure time increased.

**Table 7. Comparison of predicted and experimental toxicity**

Exposure time (min)	Toxicity as $\text{LC}_{50}$			
	$(\text{mg} \cdot \text{min}/\text{m}^3)$		$(\text{mg}/\text{m}^3)$	
	Pred.	Exper	Pred.	Exper.
20	378	430	18.9	21.5
60	493	538	8.2	9.0
180	644	899	3.6	4.9
360	762	1209	2.1	3.4
720	902	2214	1.25	3.1

Predicted values were calculated using the equation  
 $Y = -8.3461 + 3.6907 \text{Log}_{10} C + 2.7947 \text{Log}_{10} T$   
developed [1] for toxicity of GB to mice for short exposure times

## Conclusions

The inhalation toxicity values ( $\text{LC}_{16}$ ,  $\text{LC}_{50}$ ,  $\text{LC}_{84}$ ) have been estimated for GB in male CD-1 mice. All mortalities succumbed quickly. There were no apparent long term effects in the survivors.

The exposure time/toxicity relationship was not simple. It did not follow either Haber's Rule or the "toxic load" model established for exposures between 10 sec and 30 min albeit that the 20 and 60 min values obtained in this study were in excellent agreement with the historical toxicity data [1, 2] for GB in mice.

The toxicity of GB apparently decreases (in terms of the  $LC_{50}$ ) as the exposure time increases. The results suggest that a threshold value will be reached when the concentration for lethal toxicity will be minimally dependent on exposure time. A corollary to this is that there will be a lower concentration that will not produce lethal effects within a reasonable extended period. These observations are for lethal effects only. There may or may not be a similar correlation with non-lethal effects such as meiosis.

## Recommendations

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That the effects of exposure time on at least one more G agent be briefly examined to indicate that these results are not unique to GB.

That the international collaboration regarding the long term toxicity of the nerve agents be supported by the assessment of the exposure time/toxicity relationship in at least one other larger species.

That the data for all species tested be examined to attempt to get a human toxicity estimate for at least the 12 hour exposure time. This is the longest time indicated as problematical by the current military thinkers in the US, UK and Canada.

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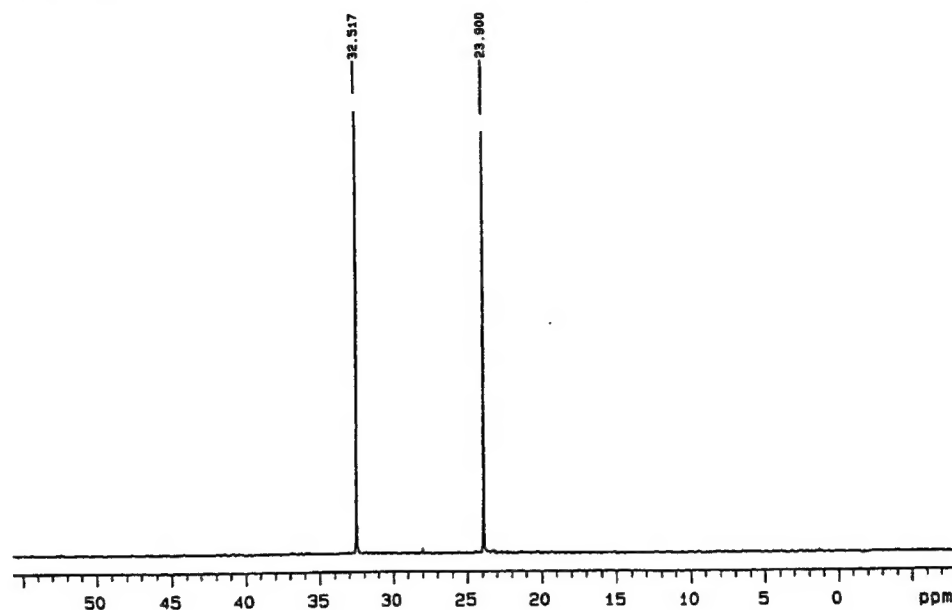
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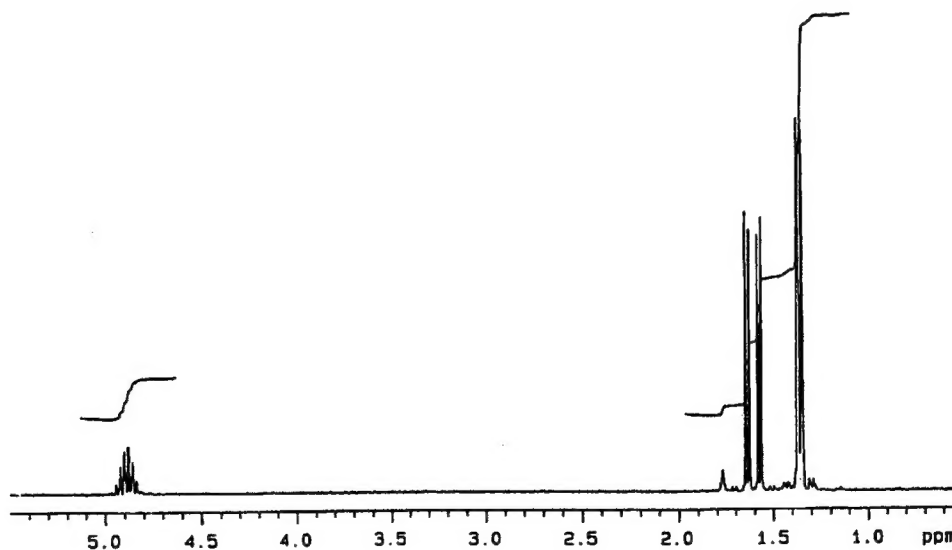
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## Annex A

### NMR spectra of GB used in this study



**Figure A1.** The  $^{31}\text{P}$  NMR spectrum of the GB used in this study shows the one doublet of P bonded to F. The indication is that the GB is >98% pure.



**Figure A2.** The  $^1\text{H}$  NMR spectrum of the GB used in this study shows that essentially all of the compound is GB confirming the >98% purity of the sample.

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<b>4. AUTHORS</b> (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)  Bide, Richard W. and Risk, Darrell J.		
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Most of the historical data for the toxicity of GB was collected for exposure times <10 min in attempts to establish the utility of and defence against this agent in offensive front line use. However, information concerning the toxicity of GB (and other nerve agents) from longer exposures of one to twelve hours is critical for personnel functioning for long periods in collective protection, for flight crew waiting for action in areas of low level contamination, for aircrew transporting previously contaminated material in cargo bays, for hospital staff who must work with contaminated victims and finally for all personnel that have been attacked, are dressed in Individual Protective Equipment and need to know when, and if, it is safe to take off these cumbersome garments.

The data presented for the toxicity of GB to mice for exposures of 20 min to 12 hours are intended to form part of an international, collaborative, multi-species effort aimed at establishing toxicity estimates for humans for these longer exposure times.  $LC_{50}$  values of 430, 538, 899, 1209 and 2214 mg.min/m<sup>3</sup> were obtained for mice for 20, 60, 180, 360 and 720 min exposures to GB, respectively. The 20 and 60 min data fit well with historical data for 20 sec to 30 min exposures. The data for longer exposures do not follow either Haber's rule ( $LC_{50} = CT$ ) or the "toxic load" expression involving  $C^aT$  previously established for 10 sec to 30 min exposures. The experimental data appear to indicate a threshold exposure concentration near 3 mg/m<sup>3</sup> for the  $LC_{50}$  for exposures up to 12 hr.

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Toxicity  
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